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Characterization of dissolved organic matter in a dynamic membrane bioreactor for wastewater treatment

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This paper systematically examined the characteristics of dissolved organic matter (DOM) in a dynamic membrane bioreactor (DMBR) for municipal wastewater with a laboratory-scale continuous-flow device. Experimental results showed that the system performed excellent pollutants' removal efficiencies. The increase of trans-membrane pressure (TMP) for the dynamic membrane (DM) could be divided into three stages, i.e., zero increase stage, slow increase stage and abrupt rise stage. The maximal fouling rate of the DM reached to 4.34 kPa/h in abrupt rise stage. It was observed that the polysaccharides (PS) concentration of DOM samples gradually increased from the anaerobic zone to the aerobic zone in sequence, but the proteins (PN) concentration performed an opposite trend. The DM could retain a small part of the large molecular substances (>10 kDa) in the aerobic zone. Two particular fluorescence peaks appeared in the anaerobic zone and in the anoxic zone were also found in the effluent, which illustrated the dynamic cake layer closed to the stainless steel mesh might induce an anaerobic/anoxic micro environment. Based on the three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy analysis, aromatic proteins, aromatic protein-like substance, fulvic acid-like substances and soluble microbial by-product-like materials could be biodegraded effectively in the DMBR, and the DM could partly remove the humic acid-like substances and soluble microbial by-product-like materials.

dissolved organic matters, dynamic membrane bioreactor, three-dimensional excitation-emission matrix fluorescence spectroscopy, membrane fouling, wastewater treatment

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The conventional membrane bioreactor (CMBR) equipped with micro-filtration membrane or ultra-filtration membrane represents an important development in the wastewater treatment and reclamation technology, but its widespread application is hampered by the high initial cost of membrane module, high energy consumption and difficult membrane fouling control [1–3]. The dynamic membrane (DM) is formed on the big aperture support mesh by the filtered solution containing fine particles [4]. Compared with the CMBR, the dynamic membrane reactor (DMBR) is advantageous in high effluent flux, easy back washing, low energy consumption and low cost of membrane module [5].

DOM originated from biological wastewater treatment process contains different organic compounds, such as carbohydrates, proteins, fulvic acid and humic acid [6,7]. Another definition is that the DOM is a heterogeneous mixture of aromatic and aliphatic organic compounds containing oxygen, nitrogen, and sulfur functional groups (e.g., carboxyl, phenol, enol, alcohol, carbonyl, amide, and thiol) [8]. DOM played an important role in membrane fouling process [9–11], and numerous efforts have been made to study the influence of DOM in mixed liquors on the membrane performance [12–14]. Kimura et al. [3] and Liang et al. [15] reported that DOM was the key membrane foulants and especially responsible for the long-term irreversible fouling of membranes. Protein-like substances in DOM was the

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dominant fluorescence substances in membrane foulants due to the membrane retention capacity [8]. Meanwhile, Liang and Song [16] found that the aquatic humic acid substances were the major component of DOM to membrane fouling. As the DMBR being a new wastewater treatment process, the information about the mutual influences of the DOM in anaerobic, anoxic and aerobic zone on the DMBR operation is limited. It is essential to study the characteristics of DOM in each reactor zone more comprehensively, as well as its potential for the dynamic membrane fouling.

Therefore, the purpose of this study is to obtain more comprehensive information on DOM in the anaerobic, anoxic and aerobic zone and their influences on the performance of the DMBR. Analytical methods including photometric quantity analysis method, gel filtration chromatography (GFC) and three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy were applied to gain insights into the characterization of the DOM samples in the DMBR. Furthermore, the effect of DOM on the DM fouling is also analyzed.

1 Materials and methods

1.1 Experimental setup

The DMBR with a total effective volume of 20 L consisted of three parts, i.e., anaerobic zone (5 L), anoxic zone (5 L) and aerobic zone (10 L) in sequence, as shown in Figure 1. The anaerobic environment was created by a water seal on the top of the anaerobic zone. The flat sheet DM module used a stainless steel mesh with an equivalent aperture of 75 μm as the support layer, and its effective filtration area was 0.036 m^2. The configuration of DM support module was referenced to Chu et al. [5]. A synthetic wastewater recipe simulating real domestic sewage was adopted. The synthetic wastewater was made by adding 150 mg/L glucose, 80 mg/L sodium acetate, 150 mg/L reptone, 80 mg/L NH_4Cl and 26 mg/L KH_2PO_4. Required trace metals provided to the bioreactor was 10.6 mg/L $CaCl_2$, 20 mg/L $MgSO_4 \cdot 7H_2O$, 3 mg/L EDTA-Na, 0.45 mg/L $FeCl_3 \cdot 6H_2O$, 0.036 mg/L $MnCl_2 \cdot 6H_2O$, 0.045 mg/L H_3BO_3, 0.036 mg/L $ZnSO_4 \cdot 7H_2O$, 0.009 mg/L $CuSO_4 \cdot 5H_2O$, 0.054 mg/L KI and 80 mg/L $NaHCO_3$ used as a buffer to adjust the influent pH. The dynamic membrane effluent was withdrawn by a peristaltic pump connected to the support module. A mercury manometer was fixed between the support module and the permeate pump to measure the TMP of the DM. Air was supplied through the pinhole aerator pipe below the membrane module to induce a cross-flow near the dynamic membrane surfaces and provide oxygen for the microorganisms in the reactor.

1.2 Operation methods

Continuous operation of the DMBR was carried out under the filtration flux of 50 L/(m^2 h). Mixed liquors of the aerobic zone and the anoxic zone were recycled to the anoxic zone (recirculation *R*) and the anaerobic zone (recirculation *r*), and the recycle rates were controlled at 2 times and 1 times of the influent flow rate, respectively. The sludge retention time (SRT) of the BDMR was maintained at 40 d by discharging the mixed liquor from the bioreactor daily. The dissolved oxygen (DO) concentration of the anaerobic zone, anoxic zone and aerobic zone was in the range of <0.2 mg/L, 0.3 mg/L and 1–2 mg/L, respectively.

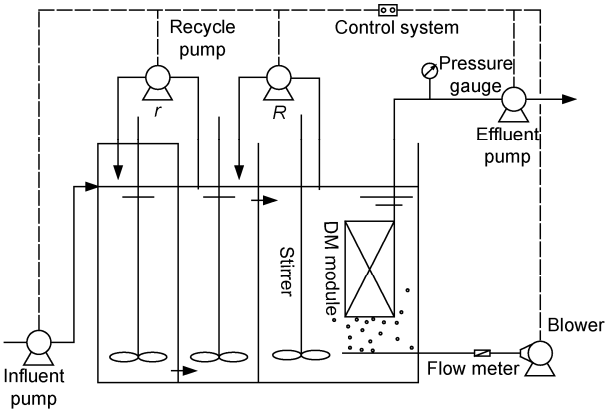

Figure 1 Diagram of the DMBR.

1.3 Analytical methods

(1) Samples pretreatment. The water phase of the activated sludge originating from the three zones of the DMBR were centrifuged (1500 r/min, 10 min) at 4°C followed by the filtration through 0.45 μm membrane. The influent and the DM effluent were filtrated through 0.45 μm membrane. All the filtrates were used as the samples for DOM analysis.

(2) Analytical items. A luminescence spectrometry (F-4500 FL spectrophotometer, Hitachi, Japan) was employed to conduct the three-dimensional EEM spectra of the all DOM samples. The software origin 8.0 (OriginLab Corporation, USA) and surfer 8.0 (Golden Software, USA) were used to process the EEM data. The filtrates of DOM samples were fractionated by a GFC analyzer, which consisted of a TSK G4000SW type gel column (TOSOH Corporation, Japan), a liquid chromatography spectrometer (LC-10ATVP, SHIMADZU, Japan) and a refractive index detector (RID-10A). Polyethylene glycols with molecular weight (MW) of 1215, 274.4, 128, 11.84 and 194 kDa (Merck Corporation, Germany) were used as standards for calibration. The total EPS dissolved in the supernatant was expressed as the sum of carbohydrates and proteins. A phenol-sulfuric acid method was used to quantify polysaccharides with a glucose standard [17]. Protein content was determined by the modified Lowry method with bovine serum albumin (BSA) as the standard [18].

Measurements of chemical oxygen demand (COD), total

nitrogen (TN), total phosphorus (TP), ammonia (NH_3-N), mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) in the reactor were performed according to the Chinese NEPA standard methods [19]. The pH was determined by a pH meter (PHS-3C, China). The dissolved oxygen (DO) concentration was measured with a DO meter (Model HQ30d, HACH, USA). The filtration flux of the dynamic membrane was measured with volumetric method.

2 Results and discussion

2.1 DMBR performance

Table 1 summarizes the regular pollutant removal efficiency in the steady operation period of the DMBR. It can be seen that the removals of pollutants were very effective by the DMBR, especially TP removal. The poly-phosphate accumulating organisms had a preferably anaerobic environment to release P in the anaerobic zone of the DMBR, and the TP concentration in supernatant of the anaerobic zone reached to around 20 mg/L, which was much greater than that in the influent. Therefore, the excessive P uptake could be achieved effectively by the poly-phosphate accumulating organisms in the aerobic zone after the completion P release in anaerobic environment. The average C:N:P ratio of 100:10.6:0.98 of synthetic wastewater and the average volumetric load of 1.08 kg COD/(m^3 d) used in this research might also facilitate the TP removal [20].

The variations of DM flux and TMP in an operation cycle are shown in Figure 2. Many studies have focused on the two-stage TMP increase of the membrane filtration process, i.e., a slow and gradual increase followed by an abrupt rise in the TMP [21–23]. According to the Figure 2, the TMP increase of the DM was divided into three stages, i.e., zero increase stage, slow increase stage and abrupt rise stage, which is similar with the findings reported by Zhang et al. [24]. The TMP increase rate per unit time of DM is directly related to the rate of membrane fouling. It can be seen that the TMP always kept at 0 kPa during the zero increase stage of the DM filtration process, which lasted approximately 50 h. The possible reason for this phenomenon might be that the cake layer deposited on membrane surface was very thin (<1 mm) at the initial filtration stage. Following closely, the

Table 1 Characteristics of the influent and effluent in the DMBR[a)]

Items	Influent	Effluent	Removal efficiency (%)
COD (mg/L)	375.8±35.5	37.1±15.8	90.0±0.04
TN (mg/L)	39.9±5.2	10.1±1.8	72.9±0.04
TP (mg/L)	3.7±0.5	0.05±0.12	98.8±0.03
NH_3-N (mg/L)	23.9±1.1	0.7±1.0	96.7±0.05

a) Values are given as mean value ± standard deviation (n=40).

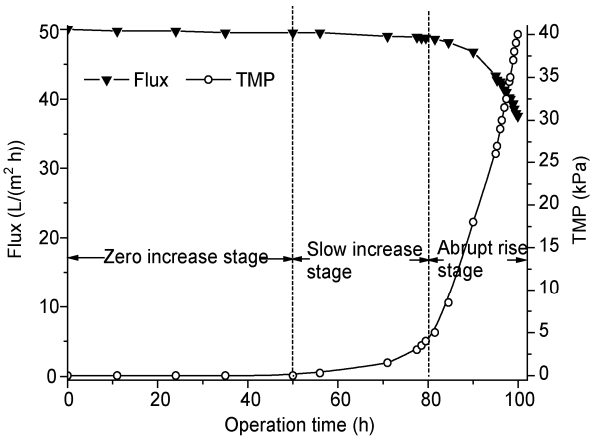

Figure 2 Variations of the flux and TMP with time in a DM filtration process.

TMP increased slowly and the average DM fouling rate was 0.16 kPa/h in this stage, which might result from the cake layer condensation and the porosity degression. Zhang et al. [24] also found that pore blocking could lead to the steady TMP rise in this stage. In the third stage, TMP increased rapidly from 5 to 40 kPa in 20 h corresponding to the maximal fouling rate 4.34 kPa/h, and the filtration flux of DM declined rapidly. The filtration process was terminated when the TMP reached 40 kPa and the thickness of cake layer on support mesh surface was approximate 0.86 cm. So the cake layer might contribute to the major filtration resistance of DM [25].

2.2 EPS concentration in DOM

EPS are complex mixture of macromolecular polyelectrolytes including polysaccharides, protein, humic compounds, and nucleic acids [26,27]. EPS, which are deemed to be the most significant biological factor responsible for membrane fouling [28], could be influenced by both the process design and the operational factors [29–31]. The variations of total EPS, PN and PS in DOM in the DMBR are shown in Figure 3(a). PN concentration decreased sharply while the concentration of PS gradually increased from the anaerobic zone to the anoxic zone and to the aerobic zone. The feeding carbon source originated from sodium acetate and glucose could be easily biodegradable. PS concentration was barely to 0.654±0.318 mg/L in anaerobic zone, and increased to 1.118±0.854 mg/L and 5.37±2.721 mg/L in the anoxic zone and the aerobic zone, respectively. Compared to the anoxic zone, the increased portion of PS in aerobic zone may be attributed to the release of microorganisms' secretion in the mixed liquid by the shear forces induced by aeration. Dvorak et al. [2] also found that the higher shear forces could cause activated sludge cells to produce more EPS to protect themselves from mechanical stress. During the treatment process, a part of PN had been degraded as essential energy for microorganism

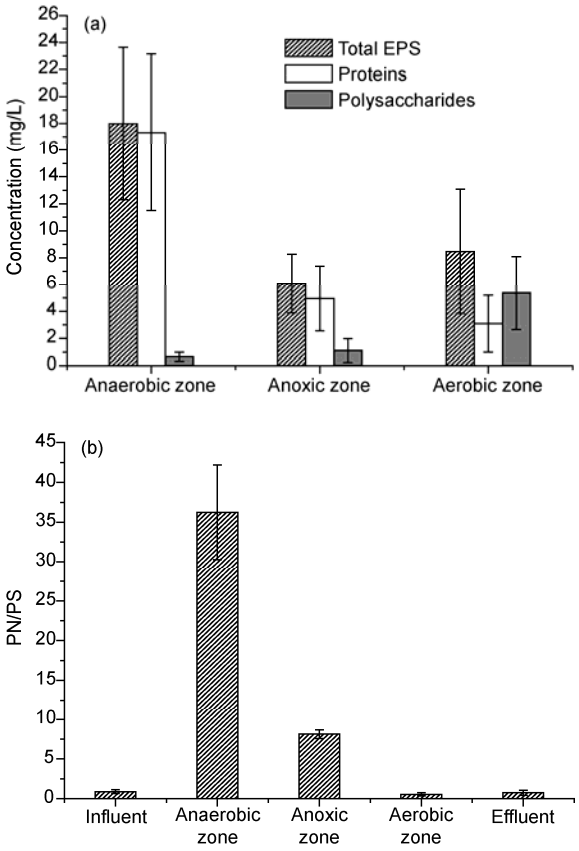

Figure 3 (a) EPS concentration in DOM; and (b) PN/PS ratio in DOM.

metabolism; meanwhile, EPS secreted by microorganism contained abundant protein-like substance, which might lead to the augmentation of PN concentration in DOM. The total EPS concentration in the aerobic zone was slightly higher than that in the anoxic zone, which was primarily influenced by the greater part of PS in the aerobic zone. So the aeration could induce more EPS released to the supernatant.

Some researchers have conducted on the membrane retention of soluble proteins and polysaccharides. Evenblij et al. [32] reported that the removal efficiencies of PN and PS by MBR were 15% and 40%, respectively. However, Drews et al. [33] achieved 20%–70% retaining of proteins and 75%–100% retaining of carbohydrates in the MBR. Figure 3b shows the variations of PN to PS (PN/PS) ratio in the DMBR of this research. The PN/PS ratio in the anaerobic zone reached as high as 36.23, and then declined gradually. The highest PN/PS ratio in the anaerobic zone might be explained that the PS (sodium acetate and glucose) could be easily degraded while some protein-like substances of PN were non-degradable by the microorganisms. The average ratio of PN/PS in the aerobic zone was slightly lower than that in the effluent, which might due to the significant retention of polysaccharides-like substances by the DM. In this research, the averaged PN retention and PS retention by DMBR was about at 70.2% and 79.6%, respectively.

2.3 Molecular weight (MW) distribution of DOM

The macromolecules or biomacromolecules substances have been widely considered as a major factor resulting in membrane fouling [34–36]. The MW distribution patterns of DOM samples were measured by the GFC method [37,38], as shown in Figure 4. Four major peak clusters in the MW order of 522.7 kDa, 32.5–52.6 kDa, 391 Da and <100 Da were observed in the influent. In the aerobic zone and the effluent, the third peak cluster with the MW of 391 Da completely disappeared, and the peak cluster with the MW of 32.5–52.6 kDa cluster notably decreased by about 20%. It can be seen that the first peak cluster in the aerobic zone and the effluent was increased than that in the influent, which might be ascribed to the biodegradation of the large molecules into small molecules by microorganisms. Moreover, the peak cluster of MW 407.8 and 71.7 kDa in the aerobic zone were higher than those in the effluent, which suggested that the DM could retain a little part of compounds with high molecular weight. The M_w/M_n (weight-average molecular weight/number-average molecular weight) of the influent, the aerobic zone and the effluent were 282.41, 451.08 and 480.21, respectively. A low coefficient of M_w/M_n indicated a narrow MW distribution of the organic substances, while soluble microbial product generated by microorganisms had a broad MW distribution [8]. So much more soluble microbial product existed in the aerobic zone than in the influent.

The percentage of size-fractionated MW was shown in Figure 5 to further elucidate the size fraction of all samples. The percentage of large molecules >100 kDa in the aerobic zone was the largest among the three tested samples. The microorganisms could secrete much EPS of strong bioactivity in the aerobic zone, which might directly contribute to the development of a cake layer and the increase of the TMP. From the aerobic zone to the effluent, the percentages of the molecules >10 kDa reduced which indicated that the DM could retain some part of large molecular substances. The macromolecular compounds can be rejected and used as substrates for microorganisms while the small-size ones

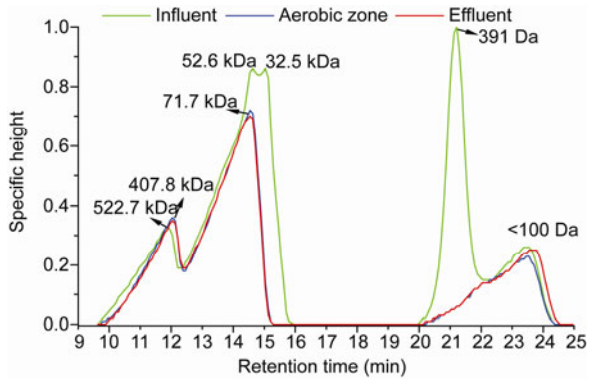

Figure 4 MW distributions of DOM samples.

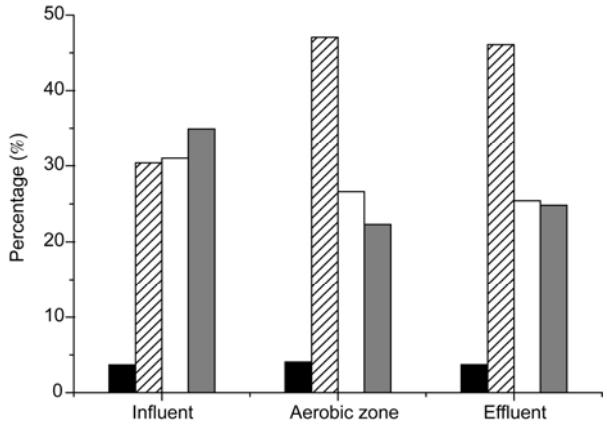

Figure 5 The percentage of size-fractionated MW of DOM samples. ■ >1000 kDa, ▨ 100–1000 kDa, □ 10–100 kDa, ■ <10 kDa.

can be discharged along with permeate in the membrane filtration process [39,40]. However, compared with the micro/ultra-filtration, the rejection degree by the DM was low. The proportions of matters with large molecular weight in supernatants and in effluents were almost identical [41].

2.4 EEM fluorescence spectra analysis

The three-dimensional EEM fluorescence spectra analysis can get spectral information about specific proteins (i.e., aromatic proteins and tryptophan proteins) and humic substances (i.e., humic acids and fulvic acids) of DOM samples [34]. The EEM fluorescence spectra of the DOM from the influent, the effluent and the aforementioned three zones in the DMBR are illustrated in Figure 6. The fluorescence intensities of the peaks in influent DOM (Figure 6(a)) were much more strongly than those in other samples (Figure 6(b)–(e)), and the peaks in the effluent DOM sample had the lowest fluorescence intensity, which is consistent with the results in Figure 3(a). The spectral peak C (E_x/E_m=230/300 nm) in the influent DOM sample disappeared in fluorescence spectra of other four samples. It also can be observed that there was no much change of the peak locations among the spectra of DOM samples from the three reaction zone in the DMBR (Figure 6(b)–(d)).

The fluorescence regional integration (FRI) method was employed to analyze the five excitation-emission regions in the EEM fluorescence spectra to better understand the information about DOM characteristics of samples [42]. Table 2 shows the EEM fluorescence spectral parameters of the DOM samples in the DMBR system. The peaks of the DOM obtained from all samples were different, which elucidated different compounds existed in the samples. According to the FRI, the regions I and II whose peaks at shorter excitation wavelengths (<250 nm) and shorter emission wavelengths (<350 nm) are related to aromatic proteins [42]. Peak C associated with the aromatic protein-like substance in the influent DOM disappeared from the anaerobic zone to the effluent. It indicates these aromatic protein-like substances which were easily biodegradable have been consumed by microorganisms in the anaerobic zone of the DMBR. The FRI of region III (E_x/E_m of 200–250/>380 nm) represented fulvic acid-like substances [42]. A new peak (peak T) at the E_x/E_m of 240/418–430 nm was appeared except in the influent samples. Therefore, certain specific fulvic acid-like organics might originate from the microorganism excretion or cell hydrolysis products. The Region IV, whose peaks at intermediate excitation wavelengths (250–280 nm) and shorter emission wavelengths (<380 nm), are related to soluble microbial by-product-like materials [42,43]. Two particular peaks at the E_x/E_m of 270/348 nm and 280/310 nm appeared in the effluent, the anaerobic zone and the anoxic zone (Table 2). The thickness of the DM gradually increased (*ca.* 15 mm at the most) as the filtration time extended, and the cake layer close to the stainless steel mesh might induce an anaerobic/anoxic micro environment. This phenomenon may facilitate the growth of anaerobic or anoxic microorganisms, and the soluble microbial by-product-like materials may enter the effluent by shear force during the filtration process. There is no obvious variations of the fluorescence intensity of peak E at the E_x/E_m of 320/396–400 nm (Region V) in all the samples. The location shift of the fluorescence peak means the considerable differences in chemical structure of DOM samples. Compared with the influent, almost all peaks of subsequent DOM samples were red-shifted (2–6 nm) and/or blue-shifted (2–6 nm) in a different degree. The red-shift and/or blue-shift of fluorescence peaks with proteins and humic substances implied the difference of compound structure in the DOM samples [44,45].

Based on the FRI method, the bar graph of the FRI percentage distribution is illustrated in Figure 7. It is interesting to note that the FRI percentage distributions of DOM samples were different. The FRI of Region V (approx.45.66%) in the aerobic zone was larger than that of any other regions, which was approximate twice as much as that of the influent (~26.54%). It demonstrates that the humic acid-like substances is the dominant matter of DOM in the mixed liquid. Meanwhile, the FRI of Region V and Region IV in the effluent were less than that of the aerobic zone, which indicated that the DM could remove part of the humic acid-like substance and soluble microbial by-product-like material. Furthermore, the difference of the FRI percentage distribution of the three zones in the DMBR should contribute to the microorganisms with different functions under various DO circumstance.

3 Conclusions

The characteristics of DOM in the DMBR were investigated. Based on this study, the following conclusions could be drawn.

The effluent quality of the DMBR was excellent during the steady-state operation, and the removal efficiency of TP

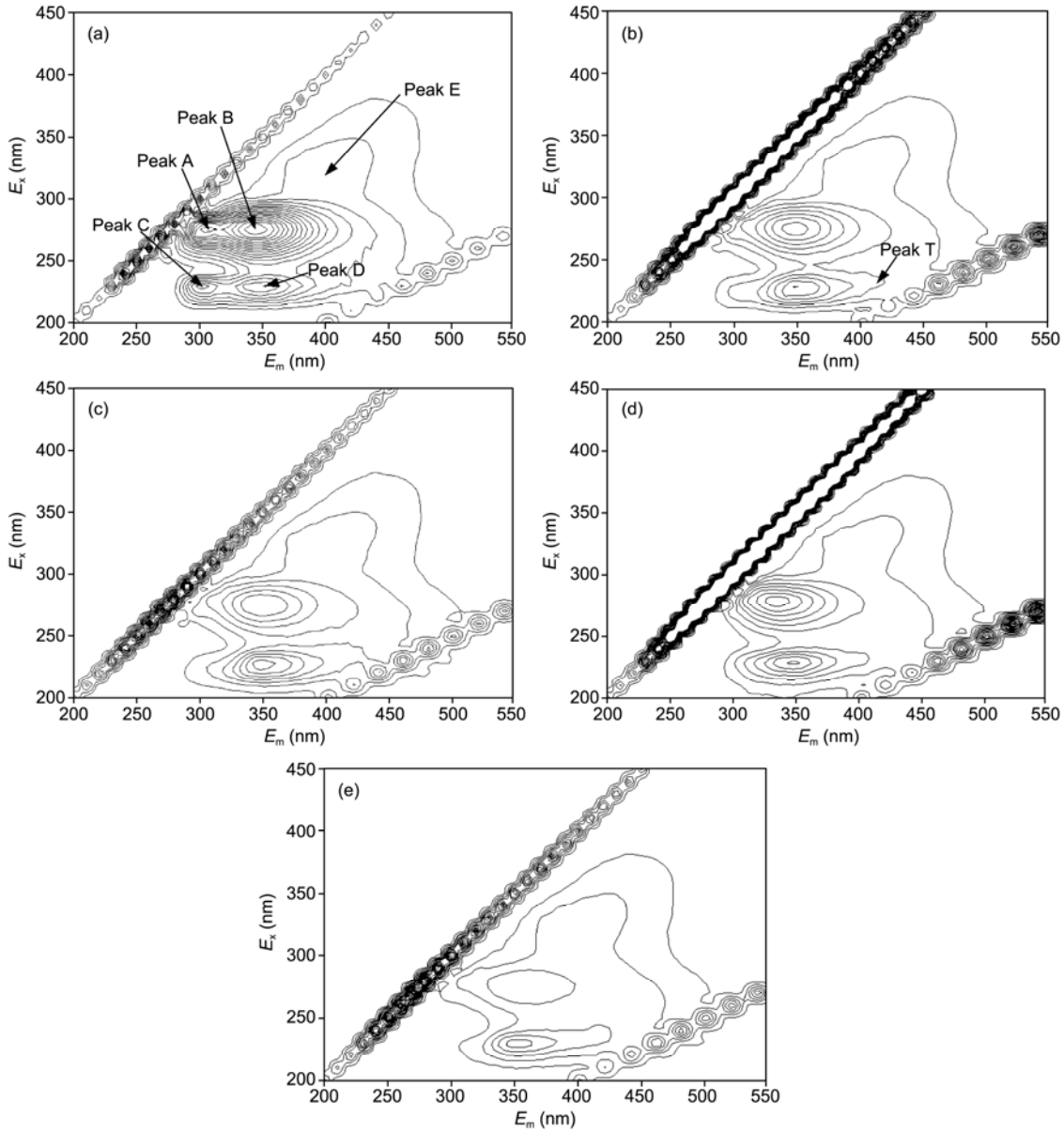

Figure 6 EEM spectra of (a) influent; (b) anaerobic zone; (c) anoxic zone; (d) aerobic zone; (e) effluent.

Table 2 Fluorescence spectral parameters of the influent, the effluent and the three zones in the DMBR[a)]

Regions	Influent		Anaerobic zone		Anoxic zone		Aerobic zone		Effluent zone	
	E_x/E_m	Intensity	E_x/E_m	Intensity	E_x/E_m	Intensity	E_x/E_m	Intensity	E_x/E_m	Intensity
I, II	230/300 230/352	225.2 245.4	230/350	209.2	230/350	227.1	230/346	224.3	230/354	177.0
III	–	–	240/422	88.3	230/430 240/428	96.4 92.0	230/428 240/424	80.8 87.5	240/418	94.5
IV	270/300 280/308 280/344	413.1 405.2 514.9	270/298 270/348 280/350	114.0 243.4 242.4	270/298 280/310 280/348	86.5 116.8 204.4	280/332	259.6	270/348 280/310	116.8 89.4
V	320/396	83.8	320/396 330/408	78.1 77.8	320/398	81.6	320/398 330/400	75.5 74.7	320/400	80.8

a) Values are given merely the fluorescence intensity higher than 70.

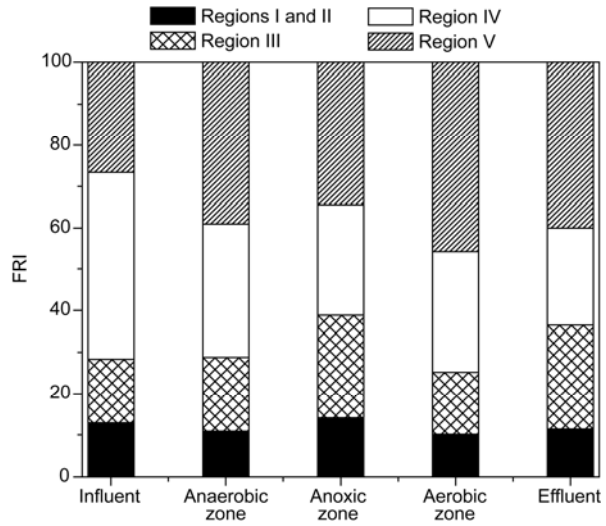

Figure 7 The bar graph of the FRI percentage distribution of the DOM samples in the DMBR.

reached to approximate 99% for synthetic wastewater. The increase of TMP for the DM could be divided into three stages.

The PS and PN concentration had opposite variation trends from the anaerobic zone to aerobic zone, and the DM rejected 70.2% proteins and 79.6% polysaccharides, respectively. The aerobic zone had a much broader MW distribution than the influent, and a small part of large molecules (>10 kDa) could be rejected by DM in the filtration process.

The aromatic protein-like substance of the influent disappeared due to biodegradation. Two particular peaks of the effluent appeared in the anaerobic zone and anoxic zone illustrated that the DM closed to the stainless steel mesh might induce an anaerobic/anoxic micro environment. The red-shift and/or blue-shift of fluorescence peaks with proteins and humic substances indicated the difference of compound structure in the DOM samples under the different operation circumstance in the DMBR.

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